

PROSTAGLANDIN PRODUCTION BY
RHEUMATOID SYNOVIAL CELLS

Stimulation by a Factor from Human Mononuclear Cells*

By JEAN-MICHEL DAYER,† DWIGHT R. ROBINSON, AND STEPHEN M. KRANE

(From the Department of Medicine, Harvard Medical School and the Arthritis Unit of the Medical Services, Massachusetts General Hospital, Boston, Massachusetts 02114)

Cultures of synovial explants from patients with rheumatoid arthritis release large quantities of collagenase and prostaglandins into the media (1-3). The collagenase can degrade undenatured collagens such as those of the joint structures, and the prostaglandins, predominantly PGE₂, may have several effects including promoting bone resorption (3, 4) and increasing local vascular dilatation and permeability (5). The cells that comprise the rheumatoid synovium can be dispersed using proteolytic enzymes, and adherent cells obtained that lack macrophage markers but produce typical mammalian collagenase for prolonged periods (weeks to >18 mo). Early in culture these cells also release large amounts of PGE₂, but secretion of PGE₂ declines rapidly after 7-10 days in culture, while collagenase production persists. Collagenase levels decline with age in culture and after passage of the cells by trypsinization and dilution (6).

Since regions of rheumatoid synovium beneath the lining cell layer usually contain large numbers of mononuclear cells (7), we reasoned, based on the work of others (8, 9), that lymphocytes and mononuclear cells might modulate the function of other synovial cells. Human mononuclear cells in culture produce a soluble factor which can stimulate the production of collagenase by the adherent rheumatoid synovial cells (10). We describe here the concomitant stimulation of PGE₂ production by the adherent synovial cells by a factor from mononuclear cells. The factor which stimulates PGE₂ production is similar in molecular weight to that which stimulates collagenase production.

Materials and Methods

Synovial Cell Culture. Isolated adherent rheumatoid synovial cells (ASC) were prepared by proteolytic dispersion of rheumatoid arthritic synovectomy specimens and cultures maintained as described previously (6, 10). Cells were passaged after treatment with trypsin-EDTA and split 1:2 or 1:4. For most experiments reported here ASC were plated in 6-cm diameter polystyrene Petri dishes at 50×10^4 cells per dish (2 ml medium) or in trays containing 24 wells of 16-mm diameter (0.3 ml medium) usually at 5 or 10×10^4 cells per well (10).

Lymphocyte Supernatant Media (LM). Heparinized venous blood from normal persons or patients with classical rheumatoid arthritis was used as a source of lymphocytes which were

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separated on sodium metrizoate/Ficoll gradients (Nyegaard and Co., Oslo, Norway) (11). The mononuclear cells were cultured at 37°C, in an atmosphere of 95% air and 5% CO₂, at 2×10^6 cells/ml in 6-cm diameter polystyrene Petri dishes in Dulbecco's modification of Eagle's medium with 10% fetal bovine serum (DMEM 10% FCS). After 24 h the nonadherent lymphocytes were separated from the adherent monocytes, centrifuged, resuspended in DMEM 10% FCS, and incubated for a further 3 days with or without phytohemagglutinin (PHA) (Wellcome Research Laboratories, Kent, England). Although most monocyte-macrophages were removed by this procedure, it was not determined to what extent the nonadherent population, here referred to as lymphocytes, was still contaminated by monocyte-macrophages. The lymphocyte-free supernatant medium (LM) prepared by centrifugation (300 *g* for 10 min) was used as a source of the stimulating factor.

Chromatography of LM. Before gel filtration, cell-free supernatant media from 9 unstimulated lymphocyte cultures and 10 cultures of lymphocytes stimulated with PHA were pooled (total vol 450 ml). Cells were from both normal and rheumatoid subjects. The pooled medium was dialyzed for 3 days against 100 volumes of 0.04% sodium azide in distilled water followed by distilled water alone. The retentate was removed, freeze-dried, and kept at -30°C until use. A solution (2.5 ml) of the dried powder in the column buffer (60 mg/ml) was then applied to a calibrated column (36.5 × 2.5 cm) of Ultrogel AcA 54 (LKB Instruments, Inc., Rockville, Md.), equilibrated, and eluted with buffer (10 mM Tris-HCl, pH 7.5, 165 mM NaCl, and 5 mM CaCl₂). The eluant fractions were diluted in DMEM 10% FCS and sterilized by Millipore filtration before incubation with ASC.

Assays. Prostaglandins in culture media were measured by radioimmunoassay utilizing an antiserum to PGE₂ (12) kindly provided by Dr. L. Levine Brandeis University, Waltham, Mass. Prostaglandins from representative culture media were further characterized by thin-layer chromatography (13). Collagenase activity was assayed using [¹⁴C]glycine-labeled guinea pig skin collagen after activation with trypsin-L-(tosylamido 2-phenyl)ethylchloromethyl ketone (Worthington Biochemical Corp., Freehold, N. J.) as previously described (6, 10).

Cell Counting and [³H]Thymidine Uptake. Cells were counted using a Coulter counter (Coulter Electronics Inc., Hialeah, Fla.). Lymphocyte [³H]thymidine uptake was measured with and without PHA (5 µg/ml) by adding 5 µCi/0.5 × 10⁶ cells/0.2 ml medium for the last 16 h of a 72 h incubation period. Isolation of ³H-DNA was performed on glass fiber strips after filtration using the MASH-II apparatus (Microbiological Associates, Bethesda, Md.).

Results

The response of production of PGE₂ and collagenase by ASC to LM in the presence and absence of indomethacin is shown in Table I. Marked stimulation by LM of both PGE₂ and collagenase was observed in the absence of indomethacin. Whereas indomethacin blocked production of PGE₂, this drug had no effect on total collagenase production. Cellular proliferation was decreased in the presence of LM but was increased when indomethacin was present in addition to LM.

It was possible to establish a dose-response for production of PGE₂ (Fig. 1 A) and collagenase (Fig. 1 B) by ASC stimulated by LM, with and without indomethacin. At all doses of LM, indomethacin blocked PGE₂ production but did not affect total collagenase production. LM prepared from cultures of lymphocytes which had been incubated in the presence of PHA had a greater stimulatory effect on production of both PGE₂ and collagenase by ASC than LM from cultures incubated without PHA (Figs. 2 A and 2 B). PHA alone at concentrations present in diluted LM incubated directly with ASC had no effect on production of either PGE₂ or collagenase.

Medium pooled from several lymphocyte cultures was chromatographed on columns of Ultrogel AcA 54 as shown in Fig. 3. The peaks of stimulatory activity for both collagenase and PGE₂ were similar and were eluted in the region between the carbonic anhydrase and ribonuclease A markers. This would sug-

TABLE I
Inhibition by Indomethacin of PGE₂ Production by Adherent Rheumatoid Synovial Cells (ASC)

	Cell number × 10 ⁻⁴ /well	PGE ₂		Collagenase	
		ng/well	ng/10 ⁶ cells	U/well	U/10 ⁶ cells
ASC	11.2 ± 0.3	0.30 ± 0.03	9.35 ± 1.27	0.020 ± 0.003	0.210 ± 0.070
ASC + indomethacin, 14 μM	11.5 ± 0.3	0.25 ± 0.01	7.21 ± 0.07	0.030 ± 0.003	0.210 ± 0.005
ASC + LM	9.2 ± 0.2	26.79 ± 1.43	946.20 ± 115.05	4.91 ± 0.59	54.58 ± 6.21
ASC + LM + indomethacin, 14 μM	14.3 ± 0.6	0.17 ± 0.01	5.48 ± 0.10	7.80 ± 0.57	56.34 ± 4.05

ASC in the second passage were plated at $\sim 9 \times 10^4$ cells per well 5 days before incubation with LM obtained from lymphocytes cultured from a normal subject. LM (0.060 ml) was added to each well containing 0.3 ml of total medium. Each value is the mean \pm SEM for four wells. Cells were counted at the end of the incubation period of 3 days. Unit (U) of collagenase activity is that which solubilizes 1 μ g of reconstituted collagen fibrils/min at 37°C.

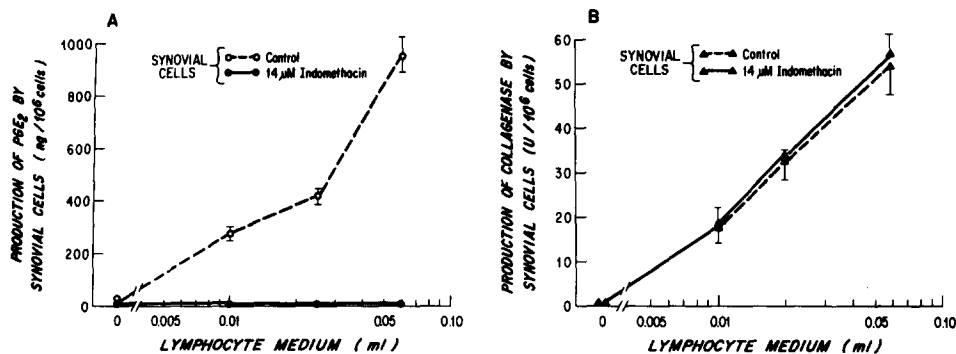


FIG. 1. Production of PGE₂ (A) and collagenase (B) by ASC as a function of the volume of added lymphocyte medium. Lymphocytes from a normal subject were incubated at 2×10^6 cells/ml for 3 days, at 37°C. The cell-free medium was added to ASC, plated 5 days previously in multi-well trays at $\sim 9 \times 10^4$ cells per well, total vol 0.3 ml, and incubation continued for 3 days at 37°C. Open symbols indicate absence and closed symbols presence of 14 μM indomethacin.

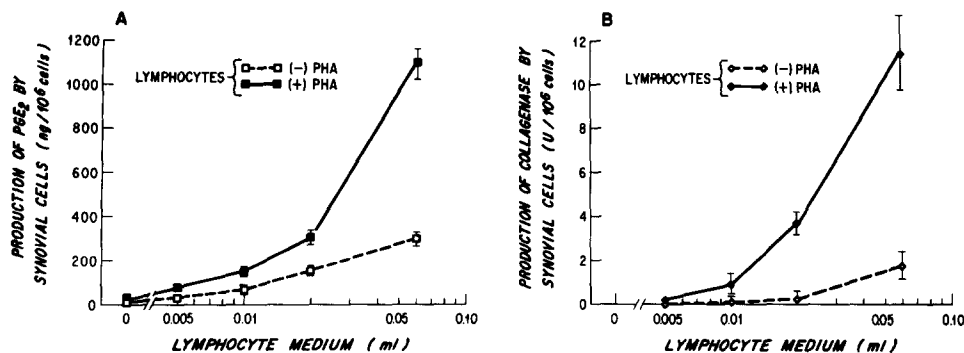


FIG. 2. Production of PGE₂ (A) and collagenase (B) by synovial cells as a function of the volume of added lymphocyte medium. Lymphocytes from a normal subject were incubated without (□, ◇) and with (■, ◆) PHA, 5 μg/ml, at 2×10^6 cells/ml for 3 days, at 37°C. The cell-free medium was added to ASC plated in multi-well trays 7 days previously at $\sim 9 \times 10^4$ cells per well, total vol 0.3 ml, and incubation continued for 3 days at 37°C.

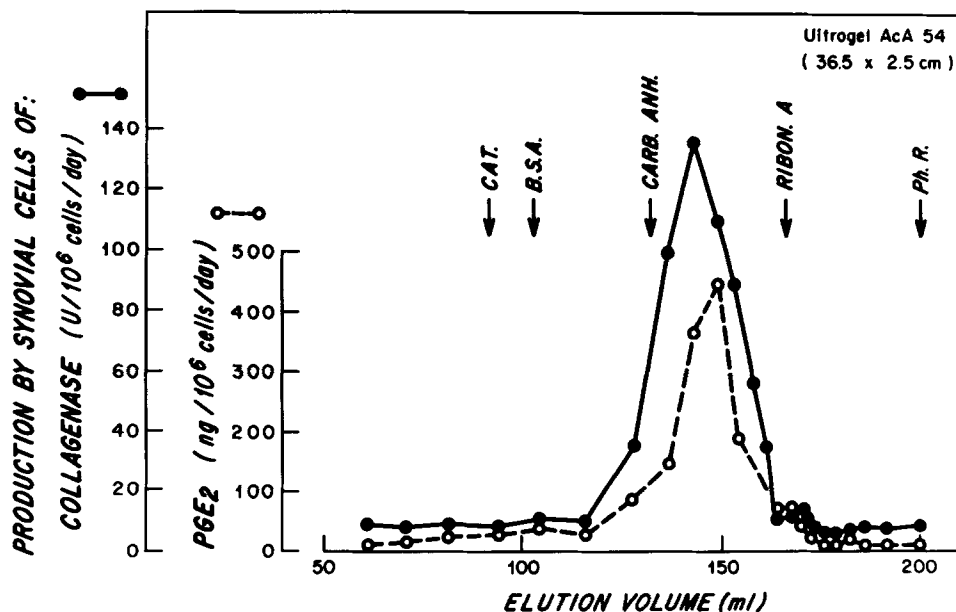


FIG. 3. Effect on ASC of lymphocyte culture medium after gel filtration. Concentrated pool of lymphocyte medium was applied to calibrated column of Ultrogel AcA 54 and eluant fractions incubated with ASC plated at $\sim 9 \times 10^4$ cells per well as described. After 3 days the media were assayed for collagenase (●) and PGE_2 (○). Arrows indicate elution position of calibration markers: CAT, catalase; BSA, bovine serum albumin; CARB. ANH., carbonic anhydrase; RIBON. A., ribonuclease A; Ph. R., phenol red.

gest an approximate molecular weight of the stimulating factor of 10,000–20,000.

The addition of lymphocyte media increased PGE release by all 20 rheumatoid synovial cell cultures so far tested. In some synovial cell cultures, even after several passages, exposure to cell-free LM increased PGE concentrations to levels approaching those found in freshly isolated synovial cells ($\sim 1,000$ ng/ 10^6 cells/day) (6). The variability in response probably depended upon several factors including passage and dilution of ASC and the source of the LM. However, each of the 45 lymphocyte preparations so far tested (from control and rheumatoid subjects) stimulated PGE production by synovial cells.

Prostaglandins from representative stimulated culture media were extracted with ethyl acetate and separated by thin-layer chromatography (13). The areas corresponding to PGE_2 and to PGA_2 and PGB_2 were identified by tritiated marker compounds, and eluted from the chromatographic strips. PGE_2 was found, in four separate stimulated cultures, to account for 85–95% of the total PG assayed (PGE_2 plus PGB_2 plus PGA_2).

Discussion

In searching for substances which might influence collagenase production by synovial cells we found that lymphocytes-monocytes in culture released a factor which markedly increased collagenase produced by these cells (10). A factor which had a similar pattern of elution from gel filtration columns also stimulated the PGE_2 production by these cells. Indomethacin, the nonsteroidal anti-inflammatory drug so far tested, inhibited PGE production but in most cultures

did not inhibit collagenase production; in some cultures even stimulation of collagenase production by LM in the presence of indomethacin was observed as was found with freshly isolated synovial cells. In the presence of lymphocyte factor, while there was a striking stimulation of the production of both PGE₂ and collagenase, there was a significant decrease in the number of synovial cells. This suppression of cell growth in the presence of the LM was reversed by indomethacin, where production of PGE₂ was also suppressed. It is possible that the decrease in cell number is due to an increase in cellular cAMP (14). In preliminary experiments we have found that PGE₂, at concentrations similar to those measured in these experiments, acutely increases levels of cAMP in these synovial cells and it is known that cAMP inhibits cell division in several different systems (15). The prostaglandins released in increased concentration stimulated by the LM could also function in a negative feedback capacity in the immune system as has been shown for several lymphokines (16).

The mechanism of the stimulation of two separate factors, collagenase and prostaglandin by the lymphocyte factor is not known. It is conceivable that the PGE₂ increase is accounted for either by increasing the availability of arachidonate or by increasing the activity of one or more enzymatic steps on the pathway of prostaglandin biosynthesis.

Summary

Human peripheral blood mononuclear cells (lymphocyte-monocyte) in culture release a soluble factor which can stimulate, up to 200-fold, production of prostaglandin E₂ by isolated, adherent, rheumatoid synovial cells. Production of the factor by the mononuclear cells is enhanced by phytohemagglutinin. This factor is similar in apparent mol wt (10,000–20,000) to that which also stimulates collagenase production by the same cells.

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